I

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: **May 5, 2009**

SUBJECT: Acibenzolar-S-methyl: Developmental Neurotoxicity Study (DNT)-Rat

PC Code: 061402
Decision No.: 208664
Petition No.: None
Risk Assessment Type: NA
TXR No.: 0052374
MRID No.: 46046401

DP Barcode: D298864
Registration No.: 100-921
Regulatory Action: Section 3
Case No.: 063611
CAS No.: 135158-54-2
40 CFR: NA

Ver.Apr.08

FROM: Gerome V. Burke, Ph.D.
Registration Action Branch 4
Health Effects Division (7509P)

THROUGH: Ray Kent
Registration Action Branch 4
Health Effects Division (7509P)

TO: Tony Kish
Fungicide Branch
Registration Division (7505P)

I. ACTION REQUESTED:

Review to confirm the data supported previous risk decisions and if MRID 46046401 is acceptable.

II. HED RESPONSE

In the developmental neurotoxicity study (MRID 46046401), the maternal LOAEL was not observed. The maternal NOAEL is 4000 ppm (326.2 mg/kg/day). The offspring LOAEL is 1000 ppm (82 mg/kg/day) based on changes in brain morphometics in the cerebellum. The offspring NOAEL is (8.2 mg/kg/day).

The HED toxicologist reviewed the DNT and the previous Risk Assessment Document submission (7/24/2000) of Acibenzolar-S-methyl. The toxicologist and risk assessor concluded that the requested DNT filled in the data gap and replaced the endpoints for Acute Dietary

Recvd in PC 6/16/200 12

(Female 13-49), Chronic Dietary (Female 13-49), Dermal Short (1-30 days) and Intermediate (1-6 months) Term, and Inhalation Short (1-30 days) and Intermediate (1-6 months) Term.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessments of motor activity and learning and memory in the offspring, and the pending review of the positive control data.

DATA EVALUATION RECORD

CGA-245704 (ACIBENZOLAR-S-METHYL)

Study Type (§83-6): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 1-01-24 (MRID 46046401)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer: David A. McEwen, B.S.	Signature: David A. McEwen Date: 4/23/09
Secondary Reviewer: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 4/23/09
Project Manager: Mary L. Menetrez, Ph.D.	Signature: Date: 4/23/09
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature: Date: 4/23/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Gerome V. Burke, Ph.D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 5/5/09
Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 5/6/09
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426

PC CODE: 061402 **DP BARCODE:** D298864
TXR#: 0052374 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): CGA-245704 (Acibenzolar-S-methyl; 97.9% a.i.; Batch #: P.303011)

SYNONYMS: S-methyl 1,2,3-benzothiadiazole-7-carbothioate

CITATION: Pinto, P.J. (2002) CGA-245704 (Acibenzolar-S-methyl): Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Study No.: CTL Number RR0930; Syngenta Number 2752-01, November 12, 2002. MRID 46046401. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, PO Box 18300, Greensboro, NC

EXECUTIVE SUMMARY - In a developmental neurotoxicity study (MRID 46046401) CGA-245704 (Acibenzolar-S-Methyl; 97.9% a.i.; Batch/Lot #: P.303011) was administered in the diet to pregnant Wistar rats (30/dose) from gestation day (GD) 7 to lactation day (LD) 22 at nominal doses of 0, 100, 1000, or 4000 ppm (equivalent to 0/0, 8.2/15.5, 82.0/153.6, and 326.2/607.8 mg/kg/day [gestation/lactation]). Dams were allowed to deliver naturally and were killed on LD 29. On postnatal day (PND) 5, litters were standardized to 8 pups/litter; the remaining offspring and dams were sacrificed and discarded without further examinations. Subsequently, 1 pup/litter/group (at least 10 pups/sex/dose when available) was allocated to subsets for FOB, motor activity, acoustic startle response, learning and memory evaluation, and neuropathological examination. Positive control data were not submitted with this study; however, summaries of positive control data previously submitted to the Agency were obtained and reviewed, and are included as an Appendix to this DER.
The maternal LOAEL was not observed. The maternal NOAEL is 4000 ppm (326.2 mg/kg/day).

In the offspring, no treatment-related effects were seen on survival, clinical signs, FOB, developmental land marks, brain weights or neuropathology. No conclusions can be drawn on

4

the effects of the compound on motor activity due the lack of consistency in the observations. At the high dose, treatment-related effects were decreased body weights and increased auditory startle response (females). Data on learning and memory were determined to be inadequate since the control values for both males and females suggest that the test animals slow with age. In addition, there was an increase in control latency rather than an expected decrease. No differences from controls were observed in any morphometric parameter in the treated animals on PND 12, except for increased ($p \leq 0.05$) length from midline in the hippocampus of the 4000 ppm females (↑8%, Table 14). The following differences ($p \leq 0.05$) in various morphometric measurements were noted on PND 63: (i) decreased dorsal cortex 1 thickness at ≥100 ppm in males (↓7-10%); (ii) decreased dorsal cortex 2 thickness at ≥100 ppm in males (↓8-12%); (iii) decreased thickness of the molecular layer of the cerebellum at the preculminate fissure in the 4000 ppm males (↓9%); (iv) decreased thickness of the molecular layer of the cerebellum at the prepyramidal fissure in the ≥1000 ppm males (↓10-15%); and (v) increased thickness of the corpus callosum in the 4000 ppm females (↑23%). The biological significance of the reduction in the dorsal cortex, which is not dose-dependent, is unclear.

The offspring LOAEL is 1000 ppm (82 mg/kg/day) based on changes in brain morphometics in the cerebellum. The offspring NOAEL is (8.2 mg/kg/day).

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessments of motor activity and learning and memory in the offspring, and the pending review of the positive control data.

COMPLIANCE - Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: CGA-245704 (Acibenzolar-S-methyl)

Description:	Yellow powder
Batch/Lot #:	P.303011
Purity:	97.9% a.i.
Compound Stability:	The test material was shown to be stable in the diet for up to 41 days at room temperature.
CAS # of TGAI:	135158-54-2
Structure:	

2. Vehicle - Diet

3. Test animals (P)

Species:	Rat	
Strain:	Wistar (Alpk:AP$_f$SD)	
Age at study initiation:	10-12 weeks	
Weight on arrival:	220-300 g (females)	
Source:	Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK	
Housing:	Dams were kept individually in solid plastic cages. The F1 animals were kept with their parent dam until PND 29 when the litters were separated by sex and housed separately (4/sex/cage) in wire mesh cages.	
Diet:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum*, except during behavioral testing.	
Water:	Tap water, *ad libitum*, except during behavioral testing.	
Environmental conditions:	**Temperature:**	22±3 °C
	Humidity:	30-70%
	Air changes:	≥15/hr
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	6 days	

B. PROCEDURES AND STUDY DESIGN

1. In-life dates - Start: 10/22/2001 End: 01/17/2002

2. Study schedule - The maternal animals were mated and assigned to study. The P females were administered the test substance continuously in the diet from gestation day (GD) 7 until lactation day (LD) 22. On postnatal day (PND) 5, the litters containing >8 pups were randomly standardized to 8 pups/litter (with equal sexes where possible) to reduce the variability. All other litters and all P females without a litter were sacrificed, and were discarded without further examinations. F_1 pups remained on study for up to PND 63 (study termination).

3. Mating procedure - The animals were mated by the breeder, and successful mating was verified by the presence of sperm in a vaginal smear. The animals were supplied on the same day that successful breeding was determined (GD 1).

4. Animal assignment - Time-mated females were randomly assigned to test groups as shown in Table 1.

Table 1. Study design [a]

Experimental Parameter	Dose (ppm)			
	0	100	1000	4000
Dams				
# of maternal animals	30	30	30	30
FOB (GDs 10, 17 & LDs 2, 9)	30	30	30	30
Offspring				
FOB (PND 5, 12, 22, 36, 46, 60)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter
Motor activity (PND 14, 18, 22, 60)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter
Auditory startle test (PND 23, 61)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter
Water maze (PND 21, 24) (PND 59, 62)	1 pup/litter 1 pup/sex/litter	1 pup/litter 1 pup/sex/litter	1 pup/litter 1 pup/sex/litter	1 pup/litter 1 pup/sex/litter
Brain weight and neuropathology [b] (PND 12) (PND 63)	1pup/litter 1pup/litter	1pup/litter 1pup/litter	1pup/litter 1pup/litter	1pup/litter 1pup/litter
Perfusion fixation, brain weight, and neuropathology (including morphometry) (PND 63)	10 pups/sex	10 pups/sex	10 pups/sex	10 pups/sex

a Data were obtained from pages 18-19 and 21-23 of the study report.
b At each sacrifice time 1 pup/litter was taken to give at least 10 pups/sex/dose.

5. Dose selection rationale - The doses presented in Table 1 were selected based on the results of a developmental neurotoxicity range-finding study (CTL study # RR0929). In this study, pregnant rats received CGA 245704 in the diet at doses of 2000, 4000, or 8000 ppm. It was stated that a dose of 8000 ppm could not be sustained through pregnancy and was terminated on GD 15/16. At 4000 ppm, slightly reduced body weight was observed in the dams on GD 22 and in the pups on PNDs 15 and 22. At 2000 ppm, reduced body weight was observed in the pups on PNDs 8 (females only), 15, and 22. No further information was provided.

6. Dosage preparation, administration, and analysis - Test diets were prepared by mixing the appropriate amount of the test material with a small amount of diet to form a premix. The premix was further diluted with diet to achieve the appropriate doses. The dams were supplied dietary admixtures beginning on GD 7 and continuing through LD 22 (inclusive). F_1 animals were not directly supplied with the test diets. Homogeneity (top, middle, bottom) was determined from samples of the 100 and 4000 ppm diets at the beginning of the study. Stability in the diet at room temperature for up to 41 days was determined using samples from the 100 and 4000 ppm dietary formulations. Concentration was determined for each dietary formulation using samples collected at the beginning of the study and once during the study.

Results - Stability (range as % of initial):
After 23 days at room temperature: 86.0-96.5%
After 41 days at room temperature: 76.8-100.7%

Homogeneity (range as % CV): 2.3-4.3% (calculated by reviewers)

Concentration (range as % of nominal):

Dose (ppm)	% of Nominal
100	96.7-97.3.0%
1000	97.9-99.0%
4000	96.6-98.1%

The analytical data indicated the mixing procedure was adequate and the variation between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals - The main study dams were checked twice daily for mortality and clinical signs of toxicity. Detailed physical examinations were recorded weekly and on PND 5. Body weights were measured on arrival at CTL, on GDs 4, 7 (prior to administration of test material), 11, 15, 19, and 22, on LDs 1, 5, 8, 12, 15, 18, and 22, and at termination (LD 29). Food consumption was recorded at intervals throughout gestation (GD 1-22) and lactation (LD 1-29), and calculated as g/rat/day.

The dams were subjected to a modified functional observation battery (FOB) outside of the home cage on GDs 10 and 17, and on LDs 2 and 9. The functional observations included, but were not limited to, the following.

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Piloerection 3) Urination and defecation 4) Ptosis 5) Exophthalmos 6) Pupillary function
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions, and general signs of toxicity (thin, altered muscle tone, dehydrated, or altered fur appearance).

b. Offspring

1) Litter observations - On PND 1 and 5, the status (sex, weight, and clinical condition) and number of all delivered pups were determined. Pups were evaluated for mortality and morbidity daily. Clinical observations were recorded daily throughout the study. Body weights were recorded on PNDs 1, 5 (precull and post-cull), 12, 18, and 22, and then weekly thereafter until sacrifice (PND 63). Post-weaning food consumption was not reported.

On PND 5, the litters containing >8 pups were randomly standardized to 8 pups/litter (with equal sexes where possible) to reduce the variability. Litters with 7-8 pups with at least 3 males and 3 females were used for F_1 evaluations; excess pups were sacrificed and discarded.

2) Developmental landmarks - Beginning on PND 41, male offspring were examined daily for preputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. The body weight on the exact days of preputial separation or vaginal patency was recorded; however, these data were not reported.

3) Post-weaning observations - After weaning on PND 22, offspring were examined for mortality and morbidity daily. Detailed physical observations and body weights were recorded weekly until sacrifice.

4) Neurobehavioral evaluations

i) Functional observational battery (FOB) - The evaluation criteria for the modified FOB were not provided. On PNDs 5, 12, 22, 36, 46, and 60, selected animals (1 pup/litter/dose) were subjected to a modified FOB in the open-field, as appropriate for the developmental stage being

observed. The same parameters assessed in the maternal FOB were examined in the offspring. The technicians were blind as to the dose group.

ii) Motor activity testing - Motor activity measurements were performed on selected animals (1 pup/litter/dose) on PNDs 14, 18, 22, and 60 using an automated activity recording apparatus (no further details provided) in a separate testing room. Data were collected in five-minute intervals over the course of 50 minutes. Total number of movements (counts) were evaluated.

iii) Auditory startle reflex habituation - Auditory startle response and habituation of responses with repeated presentation of stimuli were evaluated for selected animals (1 pup/litter/dose) on PNDs 23 and 61. The rats were tested using an automated recording apparatus (no further details provided). No details as to the duration (msec), level (dBA), or intervals of the stimulus were provided. It was not reported if any "blank" (baseline) trials were performed. The mean response amplitude and latency to the peak of the response were analyzed in 5 blocks of 10 trials each.

iv) Learning and memory testing - Learning and memory testing was performed on two sets of selected animals (1 pup/litter/dose at each time point). Water maze testing was performed with the first set of animals on PNDs 21 and 24, and a second set of animals at PNDs 59 and 62.

The water maze test consisted of 2 parts (learning ability on the first day, and memory ability 3 days later). The learning ability phase consisted of 6 trials (intervals not reported) for each rat. On each test trial, the rat was placed into the starting position (base of a Y-maze stem farthest from the two arms) and required to find the escape ladder. The scoring criteria and details of each trial were not provided. After 3 days, the memory phase was performed (6 trials for each animal) using the same animals and the same escape route. Additionally, each animal was placed in a straight channel (to measure swimming speed) after concluding the 6^{th} trial on each day.

2. Postmortem observations

a. Maternal animals - Dams that did not deliver were sacrificed, and their uteri were examined to confirm pregnancy status (no tissues were collected). Dams with total litter loss or with litters not required for F_1 selection on Day 5 were sacrificed and discarded without further examination. All other dams were sacrificed on LD 29 and discarded without further examination.

b. Offspring - All pups found dead and culled on PND 5 were discarded without further examination. Also, those animals used for neurobehavioral evaluations were sacrificed and discarded without further examination after conclusion of their respective investigations.

The animals selected for sacrifice on PND 12 (at least 10/sex/dose) were sacrificed via CO_2 asphyxiation, and the brain was immediately exposed and immersion fixed in 10% neutral buffered formalin. The brains were weighed after 24 hours fixation. The brains of the control and 600 ppm animals were embedded in paraffin, and routinely processed for microscopic evaluation.

10

On PND 63, selected animals (at least 10 pups/sex/dose) were sacrificed via CO_2 asphyxiation, and the brains were weighed prior to fixation in formalin. An additional 10 rats/sex/dose were anaesthetized with sodium pentobarbitone (i.p.), and sacrificed via perfusion fixation with modified Karnovsky's fixative. The brains were removed, weighed, and measured. The CHECKED (X) tissues listed below were removed from all animals and preserved in an appropriate fixative.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulbs	X	Sciatic nerve (proximal)
X	Frontal lobe		
X	Parietal lobe		**OTHER**
X	Midbrain with occipital and temporal lobe		Sural nerve
X	Pons	X	Tibial nerve (proximal and distal)
X	Medulla oblongata		Peroneal nerve
X	Cerebellum	X	Lumbar dorsal root ganglion
	SPINAL CORD	X	Lumbar dorsal root fibers
X	Cervical swelling	X	Lumbar ventral root fibers
X	Lumbar swelling	X	Cervical dorsal root ganglion
	OTHER	X	Cervical dorsal root fibers
	Gasserian ganglia with nerve	X	Cervical ventral root fibers
	Pituitary gland		
X	Eyes (with retina and optic nerve)		
X	Skeletal muscle (gastrocnemius)		

The central nervous system tissues, the eye (with optic nerve), and gastrocnemius muscle were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The peripheral nerves (proximal sciatic, proximal tibial, distal tibial) were embedded in resin, sectioned, and stained with toluidine blue. All tissues from the control and high dose groups, as well as the brains from the 100 and 1000 ppm males (Day 63) were examined microscopically. The morphometric evaluation was performed on various brain sections.

D. DATA ANALYSIS

1. Statistical analyses - All statistical tests were 2-sided, and significance was denoted at $p \leq 0.05$. Data were subjected to the following statistical procedures:

Parameter	Statistical test
LD 1 maternal body weight, maternal food consumption, gestation length, litter size, PND 1 mean pup body weight, total litter weight, PND 5 litter based mean body weights for selected F1 animals, motor activity measurements, maximum amplitude and time to maximum amplitude startle response, litter based time to preputial separation or vaginal patency, brain weights for selected F1 animals, brain morphometry data, and swimming times in the straight channel and individual trial times in the Y-maze	Analysis of variance
Maternal gestation and lactation body weights, mean pup body weights after PND 1, litter based mean pup body weights after PND 5, brain weights for selected F1 animals, and brain morphometry data	Analysis of covariance
Proportions of: whole litter losses, pups born live, pups surviving, litters with all pups surviving, and male pups	Fisher's Exact Test
Percentages of live born pups, pre- and post-cull pup survival, pup sex, and successful trials in the Y-maze	Double arcsine transformation of Freeman and Tukey followed by analysis of variance

Analyses of body weights, brain weights, brain morphometry data, swimming times in the straight channel, and individual trial times and percentage of successful trials in the Y-maze were performed separately for male and female pups. Analyses of *post partum* body weights and food consumption, litter size, and pup survival were presented excluding whole litter losses. The statistical analyses were considered appropriate; however, it was not reported if homogeneity of variances or normality of the data were verified. These assumptions should be verified prior to performing parametric analyses.

2. Indices - The reviewers calculated the following indices using the formulas below and included the data in the summary tables.

Fertility index (%) = $\frac{\text{\# of pregnant}}{\text{\# of females mated}} \times 100$

12

Live birth index (%) = $\frac{\text{\# of liveborn pups}}{\text{Total \# of pups born}} \times 100$

Gestation index (%) = $\frac{\text{\# of females with live pups on day of birth}}{\text{\# of females pregnant}} \times 100$

3. **Positive control data** - Positive control data were not submitted with this study; however, positive control data previously submitted to the Agency are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality, clinical signs, and functional observations** - Two dams (one each at 100 and 4000 ppm) were sacrificed prior to scheduled termination as they failed to litter. Three dams (one control and two 100 ppm) were sacrificed because of whole litter losses. All other dams with sufficient pups for F_1 selection survived to scheduled sacrifice. No treatment-related clinical signs or FOB effects were noted at any dose throughout the study.

2. **Body weight and food consumption** - No treatment-related effects on body weight or body weight gain were observed in the P females throughout the study (Table 2). The decrease ($p \leq 0.01$) noted on GD 11 (↓2%) was considered minor and not biologically important.

Table 2. Selected mean (± SD) body weights (g) for P females administered CGA-245704 in the diet from GD 7 to LD 22. [a]

Interval (Days)	Dose (ppm)			
	0	100	1000	4000
Gestation (n=29-30)				
1	257.6±15.9	260.5±17.8	259.3±16.3	259.4±14.4
11[b]	313.2	312.8	311.3	308.3** (↓2)
22[b]	410.5	413.1	414.4	411.3
Overall body weight gain (GD 1-22)[c]	149.5	154.5	156.1	152.8
Lactation (n=22-30)				
1	313.0±20.7	317.7±22.7	320.4±29.9	312.9±27.6
22[b]	363.5	367.2	363.6	365.0
29[bd]	346.9	352.9	345.0	347.6
Overall body weight gain (LD 1-22)[c]	35.5	33.5	26.2	32.1

13

a Data were obtained from pages 52-53 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.
b Adjusted means
c Values were calculated by the reviewers using the unadjusted means obtained from pages 52-53.
d Post-weaning
** Significantly different from controls at p≤0.01

No treatment-related effects on food consumption (g/animal/day) were observed in the P females throughout the study (Table 3). The differences (p≤0.05) noted at 100 ppm during GDs 15-19 (↑4%) and at 4000 ppm during LDs 15-18 (↓8%) and 22-29 (↑3%) were considered unrelated to treatment because there were no corresponding effects in body weight at these time points and/or they were not dose-dependent.

Table 3. Selected mean (± SD) absolute (g/animal/day) food consumption for P females administered CGA-245704 in the diet from GD 7 to LD 22. [a]

Interval (Days)	Dose (ppm)			
	0	100	1000	4000
Gestation (n=29-30)				
1-4	20.3±2.2	19.6±2.1	20.3±3.4	20.3±2.7
15-19	30.5±2.2	31.8±2.1* (↑4)	31.0±3.0	31.3±3.2
19-22	27.8±4.7	28.4±4.0	28.4±4.8	28.3±4.3
Lactation (n=22-30)				
1-5	33.5±7.0	31.8±6.3	31.6±5.1	33.9±7.1
15-18	63.6±3.3	63.9±4.9	62.5±6.4	58.6±4.8** (↓8)
18-22	73.5±3.9	73.7±5.7	72.8±5.6	71.3±5.3
22-29[b]	100.2±4.9	101.3±5.5	101.2±5.4	103.2±5.9*(↑3)

a Data were extracted from pages 54-55 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.
b Post-weaning
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

3. <u>Test substance intake</u> - Mean compound intake (mg/kg bw/day) during the gestation and lactation periods was calculated based on maternal food consumption, nominal dose, and body weight (Table 4).

Table 4. Mean (±SD) test substance intake (mg/kg/day) for P females administered CGA-245704 from GD 7 to LD 22. [a]

Interval	Nominal Dose (ppm)	Actual Dose (mg/kg/day)
GD 7-22	100	8.2
	1000	82.0
	4000	326.2
LD 1-22	100	15.5
	1000	153.6
	4000	607.8

14

a Data were obtained from page 148 of the study report.

4. **Reproductive performance** - All indices (fertility index, gestation length, gestation index, and incidence of dystocia) were comparable between treated and control animals (Table 5).

Table 5. Delivery observations in P females administered CGA-245704 from GD 7 to LD 22. [a]

Observation	Dose (ppm)			
	0	100	1000	4000
# of females mated	30	30	30	30
# of females pregnant Fertility index (%)	30 100.0	29 96.7	30 100.0	29 96.7
Mean (±SD) gestation length (days)	21.9±0.3	22.0±0.3	21.9±0.3	22.0±0.3
# of females with liveborn Gestation index (%)	30 100.0	29 100.0	30 100.0	29 100.0
Incidence of dystocia	0	0	0	0

a Data were obtained from pages 56 and 664-677 of the study report.

5. **Maternal postmortem results**

a. **Macroscopic examination** - The uteri of the two females that did not litter by GD 25 lacked implantation sites. No other macroscopic evaluations of the dams were performed.

b. **Microscopic examination** - No microscopic examinations were conducted on the dams.

B. OFFSPRING

1. **Viability and clinical signs** - No significant differences in live litter size, sex ratio, or post-natal survival were observed in any treated group through PND 5 (Table 6). On PND 5, all litters contained 7 or 8 animals (assumed by the reviewers); however, the mean number of pups/litter was not reported from PND 5 (post-cull) to PND 22. No treatment-related clinical signs were observed at any dose in either sex.

Table 6. F_1 live litter size and viability. [a]

Observation	Dose (ppm) 0	100	1000	4000
Number of litters	30	29	30	29
Whole litter losses	1	2	0	0
Total # of pups delivered	362	351	362	364
# of liveborn	354	346	359	363
# of stillborn	8	5	3	1
Sex ratio (% male)				
PND 1	49.4	51.1	48.7	48.2
PND 5[b]	49.6	50.7	48.9	47.9
# of deaths (PNDs 1-5[b])	14	10	13	6
Mean litter size[d]				
PND 1	11.8±2.5	12.1±3.2	12.0±3.0	12.5±3.0
PND 5[b]	11.3±2.5	11.8±3.0	11.5±2.9	12.3±2.9
PND 5[c]	NR	NR	NR	NR
PND 12	NR	NR	NR	NR
PND 18	NR	NR	NR	NR
PND 22	NR	NR	NR	NR
Live birth index (%)	97.9	98.9	99.3	99.6

a Data were obtained from pages 57-61 of the study report.
b Before culling
c After culling
d Excluding whole litter losses
NR Not reported

2. <u>Body weight and food consumption</u> - During pre-weaning days 18 and 22, body weights were decreased ($p \leq 0.05$) in both sexes at 4000 ppm (↓3-6%, Table 7a). Also, overall (Days 5-22) pre-weaning body weight gain was decreased in both sexes at 4000 ppm (↓7-8%; calculated by reviewers). Post-weaning body weight was decreased ($p \leq 0.05$) by 3% in the 4000 ppm females on PND 29 (Table 7b); however, body weights were similar between treated and control groups from PND 36-63. Overall (PND 22-63) body weight gains were similar between treated and control groups. Food consumption was not reported for the F_1 animals.

Table 7a. Selected mean (± SD) F_1 pup pre-weaning body weights and body weight gains (g). [a]

Post-natal Day	Dose (ppm)			
	0	100	1000	4000
Males				
1	5.9±0.5	6.3±0.9* (↑7)	6.1±0.5	6.1±0.6
5[bd]	10.0	9.6	9.7	9.7
5[c]	9.7±1.1	9.5±1.0	9.6±0.9	9.4±1.1
18[d]	39.9	40.7	39.5	38.7* (↓3)
22[d]	53.9	54.9	53.7	51.2** (↓5)
Overall (Days 5-22) Gain [e]	44.5	45.2	44.1	41.6 (↓7)
Females				
1	5.6±0.4	5.8±0.7	5.8±0.5	5.7±0.6
5[bd]	9.5	9.1	9.2	9.3
5[c]	9.3±1.1	9.1±0.9	9.1±0.9	9.0±1.1
18[d]	38.8	39.2	38.1	37.1** (↓4)
22[d]	52.3	52.7	51.5	49.0** (↓6)
Overall (Days 5-22) Gain [e]	43.2	43.6	42.4	39.8 (↓8)

a Data were obtained from pages 62, 69, and 70 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically. During pre-weaning, n=26-30 litters (pre-culling) or n=22-26 litters (post-culling).
b Pre-culling
c Post-culling
d Adjusted means
e Calculated by reviewers using unadjusted mean data from Days 5 (post-cull) to 22.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

Table 7b. Selected adjusted mean F_1 pup post-weaning body weights and body weight gains (g). [a]

Post-natal Day	Dose (ppm)			
	0	100	1000	4000
Males				
29	94.8	96.8	95.5	93.2
63	365.3	368.6	364.5	363.5
Overall (Days 22-63) gain [b]	311.4	313.7	310.8	312.3
Females				
29	89.9	90.6	89.1	87.4* (↓3)
36	132.4	133.3	131.9	131.0
63	225.9	227.6	224.6	225.3
Overall (Days 22-63) gain [b]	173.6	174.9	173.1	176.3

a Data were obtained from pages 69-70 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b Calculated by the reviewers using adjusted mean data.
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

3. Developmental landmarks

a. Sexual maturation - No treatment-related effect on mean time to preputial separation or mean time to vaginal patency was observed (Table 8).

Table 8. Sexual maturation (mean days ± SD) in F_1 generation rats. [a]

Parameter	Dose (ppm)			
	0	100	1000	4000
N (M/F)	91/90	76/77	90/90	90/90
Preputial separation (Males)	43.9±1.2	44.2±1.1	43.8±1.0	44.0±1.4
Vaginal patency (Females)	35.6±1.2	35.6±1.5	35.4±1.0	35.2±1.0

a Data were obtained from page 71 of the study report.

4. Behavioral assessments

a. Functional observational battery - No treatment-related behavioral effects were observed at any dose in either sex.

b. Motor activity - Overall session activity counts were increased (p≤0.05) by 45% in the 100 pm females on PND 22 (Table 9). Several isolated significant findings were noted at various intervals in both sexes throughout the study (Tables 10a and 10b). Habituation was unaffected by treatment. Overall, there is lack of consistency with the data and therefore it was determined that the motor activity data are inadequate for proper evaluation.

Table 9. Mean (±SD) motor activity data (counts) in F1 pups. [a]

Post-natal Day	Dose (ppm)			
	0	100	1000	4000
	Males			
14	198.0±155.5	110.6±98.1	122.8±126.3	113.8±104.7
18	132.8±107.1	187.6±211.5	205.7±155.1	115.7±98.8
22	323.1±161.0	326.7±236.5	266.6±134.7	258.8±132.1
60	444.5±119.8	548.7±139.3	513.8±148.0	466.9±152.5

		Females		
14	88.2±81.7	143.8±142.6	153.9±123.4	127.5±146.6
18	108.6±87.7	127.6±142.3	114.5±86.0	137.2±133.6
22	227.0±141.3	329.9±81.5* (↑45)	310.7±101.4	310.7±109.9
60	585.9±127.3	508.3±94.6	557.2±127.2	580.2±87.6

a Data were obtained from pages 72-79 of the study report; n=10-13.

Table 10a. Mean (±SD) sub-session motor activity (counts) in male F_1 pups. [a]

Interval	Dose (ppm)			
	0	100	1000	4000
	PND 14			
1-5	42.2±26.1	25.7±26.6	22.8±19.5*(↓46)	30.2±21.4
6-10	29.8±23.4	15.6±24.0	15.0±17.3	21.2±21.4
11-15	19.5±24.9	19.5±19.7	16.3±23.4	14.0±14.0
16-20	11.1±20.0	5.2±5.0	17.4±18.0	14.8±18.8
21-25	18.7±27.9	12.5±14.8	13.6±21.3	7.3±16.4
26-30	18.1±21.4	9.3±14.9	14.3±20.4	6.3±12.2
31-35	12.6±19.6	8.4±12.5	9.6±17.3	6.2±13.0
36-40	21.5±25.8	3.1±3.4**(↓86)	4.0±5.3**(↓81)	1.0±1.7**(↓95)
41-45	14.2±17.1	4.7±8.7	4.6±13.4	3.9±9.3
46-50	10.5±15.5	6.6±9.1	5.3±12.5	8.9±20.2
	PND 18			
1-5	28.3±23.7	29.7±24.0	32.6±23.7	16.7±17.9
6-10	21.3±21.2	19.5±23.9	26.3±21.6	13.3±13.7
11-15	6.7±10.4	19.7±21.5	15.5±16.6	11.0±15.4
16-20	6.2±10.2	22.3±21.3*(↑260)	20.5±23.4	11.6±17.3
21-25	12.6±25.6	21.5±24.9	11.8±19.8	2.5±5.6
26-30	8.4±13.2	17.0±24.7	12.7±19.2	9.2±13.2
31-35	15.0±19.9	17.4±24.8	21.4±22.5	15.3±21.9
36-40	10.5±15.8	16.5±25.0	23.3±22.5	15.1±20.8
41-45	12.3±18.2	14.1±23.9	21.0±25.8	9.4±15.2
46-50	11.7±16.6	9.9±21.3	20.7±19.8	11.6±19.3
	PND 22			
1-5	46.6±23.5	48.7±20.5	41.8±17.7	44.7±17.9
6-10	36.6±24.4	43.3±24.2	36.8±19.5	29.8±23.7
11-15	32.0±29.6	35.4±27.4	31.2±21.9	31.0±24.1
16-20	34.6±26.2	26.7±26.6	28.3±22.8	29.4±19.3
21-25	34.0±24.4	29.5±24.0	20.6±18.6	28.3±19.9
26-30	24.8±26.7	29.0±27.4	32.7±25.0	16.6±17.0
31-35	35.6±25.9	28.8±34.3	21.1±18.5	18.3±23.2
36-40	41.5±27.2	31.5±33.5	16.3±21.6*(↓61)	18.7±18.4*(↓55)
41-45	21.3±20.2	25.0±27.0	21.3±21.0	18.5±23.0
46-50	16.2±22.0	28.8±30.7	16.6±20.8	23.6±20.4
	PND 60			
1-5	65.5±10.2	68.3±7.8	67.8±7.9	65.0±9.5
6-10	66.1±11.8	68.2±13.9	67.0±7.0	63.8±9.1
11-15	59.4±11.0	68.9±8.5	58.8±14.4	63.7±16.1
16-20	58.8±14.8	67.0±10.4	54.4±17.2	51.8±21.0
21-25	49.7±14.1	62.4±13.8	54.4±21.1	44.0±24.9
26-30	41.8±28.7	51.6±21.1	51.6±14.7	43.5±24.9
31-35	32.7±23.3	42.9±29.0	42.8±21.7	43.6±22.0

Interval	Dose (ppm)			
	0	100	1000	4000
36-40	22.7±26.3	44.6±31.7	37.9±26.1	32.6±24.8
41-45	31.7±27.5	43.2±29.6	35.0±25.1	29.7±23.0
46-50	16.2±24.2	31.6±31.9	44.2±33.6*(↑173)	29.1±21.0

a Data were obtained from pages 72-78 of the study report; n=10-13. Percent difference from control (calculated by reviewers) is presented parenthetically.

* Significantly different from controls at p≤0.05 ** Significantly different from controls at p≤0.01

Table 10b. Mean (±SD) sub-session motor activity (counts) in female F_1 pups. [a]

Interval	Dose (ppm)			
	0	100	1000	4000
PND 14				
1-5	25.3±22.3	40.1±25.0	41.3±25.3	31.3±27.1
6-10	13.0±10.0	23.3±20.1	27.3±22.1	18.7±27.0
11-15	9.4±11.3	14.1±15.8	22.3±22.0	19.7±28.3
16-20	9.2±10.2	14.8±15.7	16.3±20.6	15.4±24.3
21-25	5.4±10.1	13.1±22.5	12.7±24.1	7.9±16.0
26-30	1.7±2.8	9.1±17.2	12.2±17.5	6.2±11.0
31-35	5.1±10.7	9.5±6.6	5.8±13.1	6.0±8.8
36-40	8.4±15.4	6.5±18.1	12.1±22.9	6.1±10.6
41-45	6.4±13.7	7.4±17.3	3.3±5.1	5.2±11.2
46-50	4.3±9.4	5.9±16.9	0.8±1.5	11.0±18.5
PND 18				
1-5	26.4±20.6	20.3±20.2	20.6±18.2	25.7±19.7
6-10	11.9±11.8	14.4±23.4	13.7±15.6	19.7±16.4
11-15	9.1±14.4	12.2±18.9	8.9±11.4	12.1±14.1
16-20	7.3±12.3	7.6±14.5	12.8±13.7	18.2±16.8
21-25	9.2±15.4	9.6±17.9	14.4±14.2	13.5±17.8
26-30	6.7±11.7	9.4±17.0	11.1±16.2	10.5±19.0
31-35	11.3±17.1	12.9±18.0	7.4±13.2	8.5±13.4
36-40	10.3±14.1	13.2±19.1	9.2±11.9	9.3±18.3
41-45	6.0±13.1	9.4±21.5	6.8±11.5	9.2±18.8
46-50	10.5±22.3	18.6±26.8	9.7±16.5	10.5±24.5
PND 22				
1-5	34.1±20.6	43.9±16.4	48.0±14.8*(↑41)	46.3±12.8
6-10	29.5±24.1	38.5±17.7	32.3±18.8	38.8±17.1
11-15	19.1±17.3	31.9±14.4	30.9±20.8	37.3±25.0*(↑95)
16-20	24.8±21.3	30.0±18.2	34.8±19.0	32.1±15.9
21-25	20.5±15.1	24.2±12.5	23.9±19.3	23.2±16.2
26-30	21.7±19.1	29.9±11.9	26.1±22.0	25.5±21.6
31-35	16.8±17.2	31.5±26.8	29.6±21.8	22.2±25.1
36-40	24.3±21.6	36.6±23.5	33.2±20.4	28.6±24.0
41-45	17.6±18.0	37.9±18.3*(↑115)	27.4±22.2	34.5±26.3
46-50	18.7±17.0	25.5±16.7	24.6±20.9	22.2±26.5
PND 60				
1-5	64.2±9.9	60.6±6.5	59.8±9.1	62.3±9.6
6-10	63.9±12.4	58.7±10.2	64.8±13.6	65.8±12.2
11-15	63.9±15.5	59.1±12.6	59.9±13.4	61.7±11.5
16-20	66.1±14.1	57.9±17.1	62.3±15.5	64.3±11.4
21-25	60.3±10.5	51.2±17.5	58.9±11.1	65.3±11.7

28

Interval	Dose (ppm)			
	0	100	1000	4000
26-30	54.5±15.7	54.5±9.4	48.3±19.1	62.8±13.1
31-35	59.5±20.8	54.7±16.4	48.9±23.0	49.8±10.8
36-40	55.5±19.8	37.8±23.1	48.6±23.6	52.8±19.1
41-45	51.3±27.1	33.7±26.6	54.5±23.7	50.5±19.5
46-50	46.7±24.3	40.1±29.4	51.3±27.0	44.8±20.8

a Data were obtained from pages 73-79 of the study report; n=10-13. Percent difference from control (calculated by reviewers) is presented parenthetically. * Significantly different from controls at p≤0.05

c. Auditory startle reflex habituation - Startle response maximum amplitude was increased (p≤0.05) in the 4000 ppm females (↑35-53%) during blocks 1-4 on PND 23 (Table 11) and in the 100 ppm males (↑58%) during block 4 on PND 61. The finding in the males was not considered treatment related because it was transient and not dose-dependent. No significant differences in maximum amplitude were observed in the males at any dose on PND 23, or in the females at any dose on PND 61. No significant differences in latency were observed in either sex on PNDs 23 or 61.

Table 11. Mean (±SD) auditory startle reflex maximum amplitude (g) in F_1 rats. [a]

Observation [b]		Dose (ppm)			
		0	100	1000	4000
Males					
PND 23	Block 1	297.1±135.6	294.7±111.4	348.9±212.0	279.1±114.5
	Block 2	234.3±63.7	254.9±80.0	239.2±69.6	209.7±74.8
	Block 3	196.9±63.7	231.2±84.7	235.4±58.7	189.8±87.7
	Block 4	185.1±60.7	201.9±76.3	205.5±70.9	177.5±63.7
	Block 5	186.3±41.0	196.9±73.6	215.7±70.8	161.3±73.3
PND 61	Block 1	695.2±195.8	939.0±369.2	762.4±280.1	861.8±356.5
	Block 2	575.2±165.9	725.8±262.5	644.9±331.5	775.5±324.8
	Block 3	540.9±184.4	651.6±234.2	567.4±256.5	624.4±329.0
	Block 4	411.5±197.6	652.1±324.5*(↑58)	557.9±312.2	575.8±234.0
	Block 5	484.2±190.3	603.9±322.1	580.5±316.2	477.5±366.4
Females					
PND 23	Block 1	218.3±43.7	295.4±114.8	224.9±60.5	333.3±134.4**(↑53)
	Block 2	213.0±52.0	243.7±89.2	199.5±70.1	304.3±128.5*(↑43)
	Block 3	199.9±54.1	212.7±74.1	183.0±64.1	270.3±64.7*(↑35)
	Block 4	182.2±53.4	186.4±47.6	155.6±61.1	246.8±60.3**(↑35)
	Block 5	177.1±55.5	186.7±56.4	158.3±45.6	200.4±54.6
PND 61	Block 1	718.4±293.1	606.8±178.3	567.8±150.6	622.2±230.2

	Block 2	585.7±198.2	570.8±214.6	591.2±219.1	567.3±207.2
	Block 3	578.5±214.5	496.6±207.6	536.9±222.9	617.5±250.4
	Block 4	487.6±263.3	428.7±190.8	475.8±214.3	504.9±222.7
	Block 5	453.3±216.2	388.5±180.7	512.2±162.1	493.7±237.8

a Data were obtained from pages 80-83; n=10-12. Percent difference from control (calculated by reviewers) is presented parenthetically.

* Significantly different from controls at p≤0.05

** Significantly different from controls at p≤0.01

b Block=10 consecutive trials

d. Learning and memory testing - No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the water maze test (Table 12). All groups showed evidence of learning (the time to complete Trial 1 was greater than the times to complete subsequent trials) and memory (the time to complete Trial 1 of the memory phase was less than the time to complete Trial 1 of the learning phase). Several differences ($p \leq 0.05$) from control were noted in the 100 ppm males and in the 1000 ppm females. However, the control values for both males and females suggest that the test animals slow with age. In addition, there was an increase in control latency rather than an expected decrease. The straight channel swimming time was increased ($p \leq 0.05$) by 139% in the 4000 ppm females on PND 62.

Table 12. Mean (±SD) water maze performance (swimming times [sec]) in F_1 rats. [a]

Parameter	Trial	Dose (ppm)			
		0	100	1000	4000
Males					
Learning (PND 21)	SC	5.83±4.22	3.71±1.77	4.21±1.72	4.36±1.91
	1	19.69±9.38	21.32±8.60	19.70±9.70	17.64±7.56
	2	12.88±7.69	10.20±5.93	14.37±5.05	9.45±3.37
	3	11.36±6.59	14.96±8.04	12.44±7.07	12.30±8.12
	4	11.53±7.25	10.14±4.20	7.86±2.87	14.33±9.00
	5	11.98±8.85	11.50±3.38	15.56±7.50	13.10±7.26
	6	10.87±5.33	9.86±5.62	9.40±4.86	10.74±4.94
Memory (PND 24)	SC	3.78±1.36	3.25±0.91	3.03±0.68	3.32±1.39
	1	7.53±3.98	5.58±2.61	8.07±3.92	7.08±3.92
	2	7.34±5.28	5.63±2.38	5.90±2.61	5.57±2.31
	3	6.82±3.35	5.71±2.77	6.42±5.85	6.34±3.46
	4	6.13±2.77	6.95±5.21	7.33±4.73	6.95±2.47
	5	5.74±2.93	9.02±3.15*(↑57)	7.27±3.74	6.15±2.51
	6	6.31±4.79	6.50±1.98	8.01±4.02	7.30±4.24
Learning (PND 59)	SC	3.22±2.17	2.42±1.01	2.50±1.18	2.60±1.01
	1	11.05±5.22	8.55±3.27	9.01±4.78	11.61±5.23
	2	6.41±2.29	4.75±2.12	6.18±4.22	6.96±5.64
	3	5.79±3.33	4.26±1.60	5.28±3.62	4.87±1.87
	4	4.03±1.50	3.80±1.38	5.38±6.63	4.75±3.01
	5	4.10±2.35	5.45±4.06	4.22±2.37	6.42±6.47
	6	3.94±1.88	4.31±2.35	4.00±1.98	5.02±2.21

Parameter	Trial	Dose (ppm)			
		0	100	1000	4000
Memory (PND 62)	SC	3.23±2.31	2.20±0.95	2.58±1.18	5.20±7.18
	1	4.80±6.66	5.87±7.62	3.66±3.42	6.35±7.25
	2	7.27±7.86	5.34±6.86	7.21±8.76	8.07±8.46
	3	9.14±8.78	7.54±5.70	8.23±7.30	6.58±7.49
	4	9.31±9.54	10.08±7.29	6.91±6.69	8.75±9.29
	5	7.01±7.44	7.85±7.66	9.07±7.57	10.40±11.42
	6	8.34±7.29	8.12±8.60	9.42±9.49	9.79±9.80
Females					
Learning (PND 21)	SC	6.67±4.75	4.42±2.82	5.51±5.45	6.05±5.06
	1	22.45±8.13	16.52±8.12	19.07±8.07	17.02±7.83
	2	12.68±8.18	13.18±6.46	16.39±7.97	12.59±5.99
	3	14.63±6.78	14.37±7.87	17.09±8.46	11.62±8.57
	4	11.55±8.21	9.02±4.63	13.33±6.84	15.39±9.60
	5	8.48±3.56	9.47±4.75	15.16±6.36**(↑79)	11.29±6.84
	6	10.89±6.04	12.35±8.82	15.02±8.36	9.51±4.40
Memory (PND 24)	SC	3.63±1.37	3.95±1.80	3.16±0.75	3.29±0.97
	1	6.58±2.29	7.48±4.12	6.33±2.31	7.29±3.15
	2	5.87±1.79	6.83±3.89	8.44±7.02	6.39±3.22
	3	6.29±4.76	7.15±3.63	7.24±3.62	6.93±4.04
	4	7.08±4.23	7.56±3.63	7.36±4.21	5.65±2.18
	5	6.36±2.65	6.44±2.77	9.39±7.03	6.05±4.54
	6	7.78±4.32	7.63±3.19	9.90±7.63	8.27±4.91
Learning (PND 59)	SC	3.00±2.77	2.33±1.08	3.02±1.51	2.54±1.19
	1	8.75±3.67	10.96±4.45	10.02±4.90	11.00±4.09
	2	6.08±3.18	6.91±3.84	5.08±1.78	6.15±3.64
	3	4.70±3.17	4.23±2.40	4.09±2.01	5.10±4.25
	4	4.41±2.50	6.86±6.89	4.82±4.06	7.13±6.74
	5	3.37±2.24	5.59±6.87	5.13±3.70	6.54±6.67
	6	4.80±4.35	6.23±7.33	5.08±2.96	7.46±7.66
Memory (PND 62)	SC	2.40±0.95	3.83±4.85	2.94±1.58	5.74±8.17*(↑139)
	1	6.75±4.79	6.33±7.59	9.21±9.34	6.45±5.69
	2	7.13±7.11	9.26±10.55	14.80±11.31*(↑108)	8.67±10.31

Parameter	Trial	Dose (ppm)			
		0	100	1000	4000
	3	10.91±9.82	15.84±11.48	12.78±11.35	13.35±10.79
	4	11.07±10.79	15.15±10.86	14.87±10.86	16.50±10.71
	5	11.25±10.12	13.29±9.89	16.02±11.77	13.76±10.66
	6	12.46±10.47	14.09±8.86	15.69±10.86	14.07±10.46

a Data were obtained from pages 88-95 of the study report; n=10-13. Percent difference from control (calculated by reviewers) is presented parenthetically.
SC Straight channel trial
* Statistically different from controls at p≤0.05
** Statistically different from controls at p≤0.01

5. Postmortem results

a. Brain weights - On PND 63, differences (p≤0.05) were observed in absolute brain weight in the 4000 ppm males (↓3%) and in the 1000 ppm females (↑3%, Table 13). However, because there was no difference in terminal body weight or relative (to body) brain weights, these findings were considered not to be treatment-related. Relative (to body) brain weights were similar between treated and control groups in both sexes throughout the study.

Table 13. Mean (±SD) absolute (g) and relative (to body, %) brain weights in F_1 rats. [a]

Parameter	Dose (ppm)			
	0	100	1000	4000
Males				
PND 12 (n=10-12)				
Terminal Body Weight (g)	24.5±2.4	24.5±1.7	24.2±1.5	24.0±1.6
Absolute Brain Weight (g)	1.13±0.07	1.15±0.07	1.15±0.06	1.15±0.05
Relative (to body) Weight (%)	4.64±0.37	4.69±0.32	4.75±0.25	4.79±0.21
PND 63 (n=10)				
Terminal Body Weight (g)	359.6±18.9	365.3±12.9	361.5±21.0	360.6±26.6
Absolute Brain Weight (g)	2.10±0.16	1.95±0.15	2.08±0.16	2.03±0.18
Relative (to body) Weight (%)	0.59±0.06	0.53±0.03	0.58±0.05	0.57±0.07
PND 63 (post-perfusion, n=10)				
Terminal Body Weight (g)	382.0±22.8	371.7±32.0	381.1±22.8	361.4±24.9
Absolute Brain Weight (g)	2.11±0.06	2.08±0.09	2.12±0.04	2.04±0.07* (↓3)
Relative (to body) Weight (%)	0.55±0.03	0.56±0.04	0.56±0.02	0.57±0.04

Females				
PND 12 (n=9-13)				
Terminal Body Weight (g)	22.2±1.8	24.1±2.2	22.9±2.0	22.6±2.1
Absolute Brain Weight (g)	1.09±0.04	1.10±0.04	1.10±0.05	1.09±0.06
Relative (to body) Weight (%)	4.94±0.37	4.58±0.40	4.84±0.37	4.84±0.42
PND 63 (n=10)				
Terminal Body Weight (g)	229.9±14.2	223.3±16.8	222.7±14.5	229.1±12.1
Absolute Brain Weight (g)	1.98±0.15	1.89±0.21	1.97±0.11	1.89±0.12
Relative (to body) Weight (%)	0.86±0.10	0.85±0.09	0.89±0.08	0.83±0.08
PND 63 (post-perfusion, n=10)				
Terminal Body Weight (g)	226.0±21.2	234.1±14.6	235.6±23.0	216.3±16.1
Absolute Brain Weight (g)	1.91±0.04	1.93±0.06	1.96±0.06* (↑3)	1.91±0.04
Relative (to body) Weight (%)	0.85±0.07	0.83±0.06	0.84±0.07	0.89±0.07

a Data were obtained from pages 104-106 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.

* Statistically different from controls at $p \leq 0.05$

b) Neuropathology

1) Macroscopic examination - Macroscopic examinations were not performed.

2) Microscopic examination - No adverse histopathological findings were noted in any group. No adverse neuropathological effects were noted in the ≥100 ppm males or in the 4000 ppm females on PNDs 12 or 63. The demyelination noted in the peripheral nerves occurred with equal frequency in the control and 4000 ppm animals. This is commonly observed in rats. No differences from controls were observed in any morphometric parameter in the treated animals on PND 12, except for increased ($p \leq 0.05$) length from midline in the hippocampus of the 4000 ppm females (↑8%, Table 14). The following differences ($p \leq 0.05$) in various morphometric measurements were noted on PND 63: (i) decreased dorsal cortex 1 thickness at ≥100 ppm in males (↓7-10%); (ii) decreased dorsal cortex 2 thickness at ≥100 ppm in males (↓8-12%); (iii) decreased thickness of the molecular layer of the cerebellum at the preculminate fissure in the 4000 ppm males (↓9%); (iv) decreased thickness of the molecular layer of the cerebellum at the prepyramidal fissure in the ≥1000 ppm males (↓10-15%); and (v) increased thickness of the corpus callosum in the 4000 ppm females (↑23%). The biological significance of the reduction in the dorsal cortex, which is not dose-dependent, is unclear.

Table 14. Mean (±SD) morphometric measurements in F_1 rats. [a]

Parameter		Dose (ppm)			
		0	100	1000	4000
PND 12					
Males					
Hippocampus	Length from midline	2.72±0.26	NE	NE	2.67±0.29
Females					
Hippocampus	Length from midline	2.63±0.20	NE	NE	2.84±0.26* (↑8)

PND 63					
Males					
Corpus callosum	Thickness	0.42±0.10	NE	NE	0.43±0.07
Dorsal cortex 1	Thickness	1.53±0.11	1.40±0.12*(↓8)	1.38±0.10**(↓10)	1.42±0.08* (↓7)
Dorsal cortex 2	Thickness	1.77±0.11	1.56±0.10**(↓12)	1.55±0.13**(↓12)	1.62±0.12* (↓8)
Cerebellum	Thickness of the molecular layer of the preculminate fissure	199.6±21.3	185.1±25.6	189.7±12.1	180.9±18.1* (↓9)
	Thickness of the molecular layer of the prepyramidal fissure	207.1±19.5	190.5±19.0	185.8±15.9*(↓10)	177.0±19.2** (↓15)
Females					
Corpus callosum	Thickness	0.35±0.06	NE	NE	0.43±0.05** (↑23)
Dorsal cortex 1	Thickness	1.46±0.11	NE	NE	1.42±0.11
Dorsal cortex 2	Thickness	1.65±0.11	NE	NE	1.58±0.13
Cerebellum	Thickness of the molecular layer of the preculminate fissure	185.2±11.7	NE	NE	195.5±21.1
	Thickness of the molecular layer of the prepyramidal fissure	200.0±23.4	NE	NE	183.4±13.3

a Data were obtained from pages 109-125 of the study report; n=9-12. Percent difference from control (calculated by reviewers) is presented parenthetically.
NE Not examined
* Statistically different from controls at p≤0.05
** Statistically different from controls at p≤0.01

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u> - The investigators concluded that treatment with CGA-245704 at up to 4000 ppm did not cause any toxicologically significant effects on the dams. In the offspring, general toxicity was characterized by decreased body weights between PNDs 18-29, increased auditory startle response in the 4000 ppm females on PND 23, and differences in morphometric measurements in both sexes on PND 63. No effects on motor activity or learning and memory were observed at any dose in either sex. No adverse neuropathological effects on various brain or peripheral nerve tissues were observed. The maternal NOAEL was 4000 ppm. The offspring NOAEL was 100 ppm.

B. REVIEWER'S COMMENTS

In the offspring, no treatment-related effects were seen on survival, clinical signs, FOB, developmental land marks, brain weights or neuropathology. No conclusions can be drawn on the effects of the compound on motor activity due the lack of consistency in the observations. At the high dose, treatment-related effects were decreased body weights and increased auditory startle response (females). Data on learning and memory were determined to be inadequate since the control values for both males and females suggest that the test animals slow with age. In addition, there was an increase in control latency rather than an expected decrease. These issues raised questions about the conduct of the memory test and confidence in the negative conclusions. No differences from controls were observed in any morphometric parameter in the treated animals on PND 12, except for increased ($p \leq 0.05$) length from midline in the hippocampus of the 4000 ppm females (↑8%, Table 14). The following differences ($p \leq 0.05$) in various morphometric measurements were noted on PND 63: (i) decreased dorsal cortex 1 thickness at ≥100 ppm in males (↓7-10%); (ii) decreased dorsal cortex 2 thickness at ≥100 ppm in males (↓8-12%); (iii) decreased thickness of the molecular layer of the cerebellum at the preculminate fissure in the 4000 ppm males (↓9%); (iv) decreased thickness of the molecular layer of the cerebellum at the prepyramidal fissure in the ≥1000 ppm males (↓10-15%); and (v) increased thickness of the corpus callosum in the 4000 ppm females (↑23%). The biological significance of the reduction in the dorsal cortex, which is not dose-dependent, is unclear.

The maternal LOAEL was not observed. The maternal NOAEL is 4000 ppm (326.2 mg/kg/day).

The offspring LOAEL is 100 ppm (82 mg/kg/day) based on changes in brain morphometics in the cerebellum. The offspring NOAEL is (8.2 mg/kg/day).

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessments of motor activity and learning and memory in the offspring, and the pending review of the positive control data.

C. STUDY DEFICIENCIES - The following deficiencies were noted:

- The evaluation criteria for the functional observational battery were not provided.
- The scoring criteria and details for the auditory startle response test and water maze test were not provided.

13544

R171018

Chemical Name: 1,2,3-Benzothiadiazole-7-carbothioic acid, S-methyl ester

PC Code: 061402
HED File Code:
Memo Date: 5/5/2009
File ID: 00000000
Accession #: 000-00-0130

HED Records Reference Center
6/19/2009